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(54) **FORMES GALENIQUES PHARMACEUTIQUES STABLES CONTENANT DE LA PARATHORMONE**

(54) **STABLE PHARMACEUTICAL FORMS OF ADMINISTRATION CONTAINING PARATHYROID HORMONE**

(57) L'invention concerne des compositions pharmaceutiques de parathormone stables au stockage, qui contiennent un aminoacide basique et éventuellement un acide organique ou inorganique présentant une bonne tolérance sur le plan physiologique.

(57) The invention concerns pharmaceutical preparations of parathormone. The preparations are stable during storage and contain a basic amino acid and optionally an organic or inorganic physiologically tolerated acid.

Stable pharmaceutical forms of administration containing parathyroid hormone

The invention concerns pharmaceutical preparations which contain parathyroid hormone or fragments thereof as the active substance as well as corresponding pharmaceutical forms of administration in the form of lyophilisates or injection solutions.

Parathyroid hormone (PTH) is a protein composed of eighty-four amino acids which is involved in the regulation of the calcium and phosphate content of blood and tissue. It is known from the literature (cf. also WO 90/10067; WO 91/06564; EP 0 301 484; WO 93/15109) that N-terminal fragments of this hormone and also peptides with appropriate modifications of the amino acid sequence have an analogous biological activity to PTH(1-84).

However, due to oxidation processes at the free methionine groups in the molecule, it is difficult to stabilize PTH in a pharmaceutical form of administration. Addition of antioxidants such as methionine or ascorbic acid does not lead to forms of administration with an adequate stability for pharmaceutical purposes. It is known from EP 0 619 119 that a stabilization can be achieved by freeze-drying a combination of sugars and sodium chloride. However, it has been shown that this type of stabilization favours the formation of dimers. However, dimers are problematic in pharmaceutical forms of administration since they can

lead to undesired side-effects when administered to patients due to immunological reactions. In addition dimers can lead to a loss of activity of the protein in the form of administration especially when the forms of administration are stored over a long time period and not at optimal temperatures. In addition lyophilization is problematic when manufacturing appropriately dried pharmaceutical forms of administration.

It was surprisingly found that stable pharmaceutical forms of administration of PTH or fragments thereof are obtained if one or several basic amino acids, in particular arginine, lysine or ornithine, are contained in the form of administration as pharmaceutical auxiliary agents. As a result it is possible to omit the addition of antioxidants or tensides. Moreover, the addition of basic amino acids leads to forms of administration which are stable on storage over a longer period of time. In particular the undesired formation of aggregates can be reduced or substantially avoided. In addition it was found that the lyophilization can be improved by the further addition of an acidic amino acid and/or a neutral amino acid. Stable aqueous forms of administration are in particular those which contain an inorganic or organic acid and have a pH value of 4 to 8, in particular 6 to 8.

Parathyroid hormone fragments that come into consideration within the sense of the invention are in particular the human N-terminal fragments of the intact protein for example the fragments (1-34), (1-35), (1-36), (1-37) or (1-38). However, it is also equally possible to use those fragments of parathyroid hormone which are N- and C-terminally shortened such as fragments which are shortened by one or two amino acids at the N-terminus. In

addition appropriate variants or modifications of this hormone can be used in which one or several amino acids are substituted by other amino acids in the amino acid sequence of PTH (1-84). This also applies to the corresponding N- and/or C-terminally shortened fragments. In particular within the sense of the invention pharmaceutical preparations can be produced which contain the parathyroid hormone fragment hPTH 1-37. This fragment is very labile due to its structure and conformation. Therefore the production of pharmaceutical preparations containing conventional pharmaceutical auxiliary substances often leads to instabilities of the products since the content of active substance is continuously decreased by agglomeration or decomposition of the peptide during longer storage.

The content of the active substance parathyroid hormone or parathyroid hormone fragments in the liquid forms of administration is up to 10 mg/ml, in particular up to 5 mg/ml or up to 2 mg/ml. The content of active substance is preferably at least 0.01 mg/ml, 0.04 mg/ml or 0.1 mg/ml. The content of active substance is more preferably 0.01 - 5 mg/ml in particular 0.04 - 2 mg/ml. The lyophilized forms of administration contain the active substance in amounts that enable corresponding infusion or injection solutions of the active substance in the said concentration ranges to be obtained by adding a certain volume of a physiologically tolerated reconstitution solution. The pharmaceutical forms of administration within the sense of the present invention are essentially free of tensides and conventional physiologically tolerated antioxidants in particular of reagents containing sulfhydryl groups such as for example methionine or cysteine or ascorbic acid. Moreover they are preferably essentially free of chloride ions since

chloride ions favour the formation of dimers. In particular the forms of administration according to the invention do not otherwise contain any other pharmaceutical additives or auxiliary substances such as e.g. sugars or physiologically tolerated polymers. The forms of administration within the sense of the invention are characterized in particular by being composed essentially only of amino acids and organic or inorganic acids but they contain at least one basic amino acid.

All physiologically tolerated amino acids with at least one basic side group come into consideration as basic amino acids within the sense of the present invention. Basic side groups are in particular amino groups which can be optionally substituted by other residues such as e.g. C_1-C_6 alkyl groups. In particular histidine, lysine, arginine or ornithine come preferably into consideration as basic amino acids.

Correspondingly physiologically tolerated amino acids with side groups which do not have sulfhydryl groups (cysteine, methionine) come into consideration as neutral amino acids such as phenylalanine, glycine or isoleucine.

The amino acids can in principle be used in the form of their racemates or their optically active forms (D or L amino acids). The concentration of the amino acids in the liquid form of administration is in the range up to 100 mg/ml. Concentrations of up to 80 mg/ml, in particular up to 60 mg/ml and up to 50 mg/ml or 40 mg/ml are particularly advantageous. The amino acid concentration is advantageously at least 1 mg/ml, in particular at least 5 mg/ml.

Within the sense of the present invention physiologically tolerated carboxylic acids, hydroxy-carboxylic acids or amino acids as well as salts thereof, especially alkali salts, come into consideration as organic acids. Lactic acid, acetic acid, citric acid or aspartic acid are advantageous within the sense of the invention. If these acids have a chiral centre, the racemates and also the optically active derivatives can be used.

Suitable inorganic acids are physiologically tolerated acids such as phosphoric acid or sulfuric acid or salts thereof which can also be used as a buffer in aqueous solution such as sodium dihydrogen phosphate, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydrogen sulfate etc.. The organic or inorganic acids can also be used in combination with one another. The amount of acid is selected such that the aqueous solution has a pH value of 4 - 8, preferably 6 - 8. As a rule the concentration of the acid in the solution is up to 100 mg/ml, in particular up to 50 mg/ml or up to 40 mg/ml. The concentration of the acid is at least 1 mg/ml, preferably at least 5 mg/ml or 10 mg/ml.

Physiologically tolerated auxiliary substances that are preferably used within the sense of the present invention are in particular the following combinations of additives: a) arginine and phosphoric acid (arginine phosphate), b) arginine, phosphoric acid and aspartic acid or c) arginine, phosphoric acid, aspartic acid and isoleucine.

The pharmaceutical forms of administration can be provided as ready-to-inject injection or infusion

solutions in appropriate ampoules. Alternatively it is also possible to provide appropriate lyophilisates which can be converted into the aqueous form by addition of isotonic solvents shortly before administration to patients.

It is expedient to produce the forms of administration by producing an aqueous solution of all constituents and transferring them into appropriate ampoules or glass vials. In the case of lyophilisate production they are preferably dried directly in the glass containers into which the solution was filled.

The invention is elucidated in more detail by the following examples of application.

Example 1

15 mg PTH (1-37) and 2.5 g L-arginine were dissolved in 100 ml water for injection purposes and the pH value was adjusted to pH 6.5 with 99 % acetic acid. This solution was sterilized by filtration and 1 ml aliquots of this solution were dispensed into glass vials under nitrogen gassing.

Example 2

15 mg PTH (1-37) and 5.5 g arginine were dissolved in 100 ml water for injection purposes and the pH value was adjusted to pH 7.4 with 85 % phosphoric acid. This solution was sterilized by filtration and 1 ml aliquots

of the solution were dispensed into glass vials under nitrogen gassing. These vials were lyophilized, sealed and crimped.

Example 3

150 mg PTH (1-37) and 5.5 g arginine were dissolved in 100 ml water for injection purposes and the pH value was adjusted to pH 7.4 with 85 % phosphoric acid. This solution was sterilized by filtration and 1 ml aliquots of the solution were dispensed into glass vials under nitrogen gassing. These vials were lyophilized, sealed and crimped.

Example 4

15 mg PTH (1-37), 3 g L-arginine and 2 g aspartic acid were dissolved in 100 ml water for injection purposes and the pH value was adjusted to pH 7.4 with 85 % phosphoric acid. This solution was sterilized by filtration and 1 ml aliquots of the solution were dispensed into glass vials under nitrogen gassing. These vials were lyophilized, sealed and crimped.

Example 5

15 mg PTH (1-37), 3.0 g L-arginine and 2.0 g isoleucine were dissolved in 100 ml water for injection purposes and the pH value was adjusted to pH 7.4 with 85 % phosphoric acid. This solution was sterilized by filtration and 1 ml aliquots of the solution were dispensed into glass vials under nitrogen gassing. These vials were lyophilized, sealed and crimped.

Example 6

15 mg PTH (1-37), 3.0 g arginine, 1.0 g aspartic acid and 1.0 g isoleucine were dissolved in 100 ml water for injection purposes and the pH value was adjusted to pH 7.4 with 85 % phosphoric acid. This solution was sterilized by filtration and 1 ml aliquots of the solution were dispensed into glass vials under nitrogen gassing. These vials were lyophilized, sealed and crimped.

Example 7

15 mg PTH (1-37), 2.0 mg sucrose and 100 mg sodium chloride were dissolved in 100 ml water for injection purposes. This solution was sterilized by filtration and 1 ml aliquots of the solution were dispensed into glass vials under nitrogen gassing. These vials were lyophilized, sealed and crimped.

Example 8

In this example the formulation of example 2 was prepared with various pH values. The amount of arginine is varied simultaneously.

Formulation	Arginine	pH value
8 a	5.8 g	8.0
8 b	5.0 g	6.5
8 c	3.2 g	5.0
8 d	3.2 g	3.0

PTH and arginine were dissolved in 100 ml water for

injection purposes and the pH value was adjusted to the respective pH value with 85 % phosphoric acid. The solutions were sterilized by filtration and 1 ml aliquots of the solutions were dispensed into vials under nitrogen gassing. These vials were lyophilized, sealed and crimped.

Example 9

The formulations in example 9 were prepared analogously to the formulation of example 2. In formulation 9a) 10 mg methionine was used. In formulation 9b) 10 mg ascorbic acid was added. Both solutions were adjusted to a pH value of 7.4. The solutions were sterilized by filtration and 1 ml aliquots of the solution were dispensed into vials under nitrogen gassing and these vials were lyophilized, sealed and crimped.

Example 10

The formulation of example 2 was used in example 10 with addition of 10 mg Tween 20. In this case PTH (1-84), arginine and Tween 20 were dissolved in water for injection purposes and the pH value was adjusted to pH 7.4 with 85 % phosphoric acid. This solution was sterilized by filtration and 1 ml aliquots of the solution were dispensed into vials under nitrogen gassing. These vials were lyophilized, sealed and crimped.

Example 11

15 mg PTH (1-37) and 3.5 g histidine were dissolved in

100 ml water for injection purposes and the pH value was adjusted to pH 7.4 with 85 % phosphoric acid. This solution was sterilized by filtration and 1 ml aliquots of the solution were dispensed into vials under nitrogen gassing. These vials were lyophilized, sealed and crimped.

Example 12

15 mg PTH (1-84) (manufacturer Sigma Corporation) and 5.5 g L-arginine were dissolved in 100 ml water for injection purposes and the pH value was adjusted to pH 7.4 with 85 % phosphoric acid. This solution was sterilized by filtration and 1 ml aliquots of the solution were dispensed into vials under nitrogen gassing. These vials were lyophilized, sealed and crimped.

Notes on the table:

The results of the examination of stability after a storage period of one or three months at refrigerator temperature and at 50°C are summarized in Table 1 for the above-mentioned examples of application.

No dimers were found when determined by the SDS-PAGE method in the forms of administration according to the invention. In addition it turned out that the PTH content was at least 98 % after a storage period of one or three months at ca. 4°C. Even at higher temperatures (50°C) no significant loss of the PTH content was found.

In contrast the forms of administration described in

examples 7, 9 and 10 favour the formation of dimers and show a lower PTH content after a storage period of one or three months and can therefore be ranked as less suitable pharmaceutical forms of administration in relation to storage stability. In principle it has turned out that the addition of chloride ions has a negative effect on the storage stability so that chloride-free forms preferably come into consideration in the forms of administration according to the invention.

Table:

Results of the examination of stability after 3 months

Example	PTH content	basic AA/ sugar	org/inorg. acid	neutr. AA/other components	pH	PTH content % (RP-HPLC)					dimers (SDS-Page)
						Start	1 month 4°	1 month 50°	3 months °	3 months 50°	
1	0.15 mg	arginine	acetic acid	-	6.5	99.2	98.6	92.4	98.2	85.7	-
2	0.15 mg	arginine	phosphoric acid	-	7.4	99.0	98.9	98.5	98.2	98.5	-
3	1.50 mg	arginine	phosphoric acid	-	7.4	99.2	99.1	98.7	98.7	98.5	-
4	0.15 mg	arginine	phosphoric acid/ aspartic acid	-	7.4	99.9	97.8	98.1			-
5	0.15 mg	arginine	phosphoric acid	isoleucine	7.4	98.6	98.2	96.4			-
6	0.15 mg	arginine	phosphoric acid/ aspartic acid	isoleucine	7.4	99.3	99.1	98.4	98.2	96.6	-
7	0.15 mg	sucrose	-	NaCl	5.1	98.0	98.9	98.6	98.9	98.6	++
8	0.15 mg	arginine	phosphoric acid	-	8.0	97.7	98.5	97.4			-
8	0.15 mg	arginine	phosphoric acid	-	6.5	98.0	98.6	97.1	98.1	96.7	-
8a	0.15 mg	arginine	phosphoric acid	-	5.0	98.6	98.4	96.8			-
8b	0.15 mg	arginine	phosphoric acid	-	3.0	98.2	98.1	96.4			-
9a	0.15 mg	arginine	phosphoric acid	methionine	7.4	97.5	96.7	96.3			+
9b	0.15 mg	arginine	phosphoric acid	ascorbic acid	7.4	92.9	89.8	85.9			++

amended claims

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Claims

1. Storage-stable pharmaceutical preparation in the form of a lyophilisate or an injection or infusion solution containing parathyroid hormone or a parathyroid hormone fragment as the active substance, wherein the preparation contains one or several basic amino acids and is essentially free of physiologically tolerated antioxidants.

2. Pharmaceutical preparation as claimed in claim 1, additionally containing an organic or inorganic acid in an amount which is suitable for adjusting the pH value of the injection or infusion solution to a range of pH 4 to 8.

3. Pharmaceutical preparation as claimed in claim 1 or 2, wherein it additionally contains one or several neutral amino acids.

4. Pharmaceutical preparation as claimed in one of the claims 1 - 3, wherein the basic amino acids are histidine, lysine or arginine.

5. Pharmaceutical preparation as claimed in one of the claims 1 - 4, wherein the organic acid is selected from the group of carboxylic acids or amino acids in particular lactic acid, acetic acid, citric acid or aspartic acid.

6. Pharmaceutical preparation as claimed in one of the claims 1 - 4, wherein the inorganic acid is phosphoric acid or sulfuric acid.

7. Pharmaceutical preparation as claimed in one of the claims 1 - 6, wherein it contains combinations of organic and inorganic acids in particular phosphoric acid and aspartic acid or physiologically tolerated salts thereof.

8. Pharmaceutical preparation as claimed in one of the claims 3 - 7, wherein it contains sulfhydryl group-free amino acids especially phenylalanine, glycine or isoleucine as neutral amino acids.

9. Pharmaceutical preparation as claimed in one of the claims 1 - 8 in the form of a liquid form of administration for injection or infusion purposes, wherein the pH value of the solution is in the range of 4 to 8.

10. Pharmaceutical preparation as claimed in one of the claims 1 - 8 in the form of a lyophilisate for the preparation of an infusion or injection solution having a pH value of 4 to 8 in particular with a pH value of 6 to 8.

11. Pharmaceutical preparation as claimed in one of the claims 1 - 10, wherein it contains the active substance in a concentration amount of 0.01 to 10 mg/ml preferably of 0.04 to 2 mg/ml.

12. Pharmaceutical preparation as claimed in one of the claims 1 - 11, wherein it contains the basic amino acid at a concentration of 1 - 100 mg/ml preferably of 5 - 60 mg/ml.

13. Pharmaceutical preparation as claimed in one of the claims 1 - 12, wherein it contains the organic or inorganic acid at a concentration of 1 - 50 mg/ml preferably of 5 - 40 mg/ml.

14. Pharmaceutical preparation as claimed in one of the claims 1 - 13 essentially composed of amino acids and an organic or inorganic acid wherein it contains at least one basic amino acid.

15. Process for the production of pharmaceutical preparations as claimed in one of the claims 1 - 14, wherein a solution or suspension of the active substance is produced in a physiologically tolerated solvent and one or several basic amino acids and one or several organic or inorganic acids are added and subsequently the solution is processed to ready-to-inject injection of infusion solutions or the solution is lyophilized.

16. Use of pharmaceutical forms of administration as claimed in one of the claims 1- 14 for the production of pharmaceutical preparations for the treatment of diseases of calcium metabolism in particular of osteoporosis.

17. Use of a basic amino acid for the production of storage stable pharmaceutical forms of administration containing parathyroid hormone or a parathyroid hormone fragment, wherein the forms of administration are essentially free of physiologically tolerated antioxidants.